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THE HOUSE-FLY MYCOSIS CAUSED BY *ENTOMOPHTHORA MUSCAE*: INFLUENCE OF RELATIVE HUMIDITY ON INFECTIVITY AND CONIDIAL GERMINATION

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Abstract.—Studies on the effects of various relative humidities on the infectivity of *Entomophthora muscae* conidia showed that *Musca domestica* exposed to conidial showers falling through atmospheres with relative humidities ranging from zero to 100% acquired fatal infections. Studies on the germination of conidia held at various humidities showed that the fungus produces long germ tubes only from secondary conidia at 100% R.H. These results suggest that *E. muscae* can be transmitted within fly populations during periods of dry-cool weather, and that the relative humidity within the boundary layer surrounding the fly's body is at or near the saturation point. Temperature maintained during the studies was 21 ± 3°C.

Introduction

The mycosis of the house fly caused by *Entomophthora muscae* has been recognized for more than a century. One factor that undoubtedly influences the occurrence of this disease in populations of *Musca domestica* and other species is atmospheric humidity. Wilding and Lauckner (1974), in a review of the literature pertaining to this subject, noted that epizootics occur mainly in wet and cool periods but are known from dry and warm ones as well. In their own studies these authors found little correlation between atmospheric conditions and the incidence of the disease in the field. Berisford and Tsao (1974) also found no striking relationship between the incidence of *E. muscae* infections and weather conditions. Baird (1957) noticed accidental transmission of *E. muscae* among caged flies held in the laboratory at about 23°C and a relative humidity of 50%; for reasons unknown the fungus was not transmissible after the cultures of flies were transferred to another room with similar atmospheric conditions. I (Kramer, 1980) reported successful transmission of *E. muscae* to house flies held in humid chambers at 14–27°C and no transmission of this pathogen to flies held in a dry chamber at 21–27°C. I also noted that many conidia held on glass slides in a humid chamber germinated whilst few conidia held under similar circumstances in a dry chamber did so. The present study defines with greater precision the effect of humidity in the ambient environment on the infectivity of *E. muscae* conidia and on the germination of *E. muscae* conidia.

Materials and Methods

I collected about 30 living cluster flies *Pollenia rudis* from the window panes of a heated building where a natural epizootic of the mycosis was in progress on December 8, 1979. Cluster flies from this collection dying of the disease over the next six days served as a source of inoculum used to establish *in vivo* cultures of *E. muscae* in house flies by a method described previously (Kramer 1980). Conidia-bearing cadavers from these cultures served as the source of inoculum for the studies described herein. Primary conidia were uniformly bell-shaped with an apical point while the smaller secondary conidia lacked the apical point; clearly the fungus was *E. muscae* (see MacLeod et al. 1976).

Infectivity Tests.—Batches of 20 to 25 young healthy flies were placed in cylindrical, clear plastic cages (height 35 mm, width 30 mm) having two large mesh-covered windows and mesh-covered tops and bottoms. Three fresh cadavers of *M. domestica* with *E. muscae* infections were affixed with petrolatum to the inner surface of small plastic dishes. A dish bearing cadavers was placed over each cage to allow the conidia discharged to fall through the mesh-covered top into the cage of flies. These cages with dishes were placed in glass battery jars tightly covered with layers of polyethylene wrap and waxed paper. The relative humidities maintained in these chambers are given in Table 1. After 24 hours the test flies were fed honey and the dishes with cadavers were removed. After an additional 24 hours the flies were transferred to glass-covered carton cages, and each cage was provisioned with a water bottle and a mixture of dried milk and sugar. These cages were held at 21 ± 3°C and 45% R.H. with a 18:6 photoperiod. The cages were checked for infected flies at daily intervals. The findings are given in Table 1.

Germination of Conidia.—Three or four freshly dead or dying house flies with *E. muscae* infections were affixed to the inner surface of small plastic dishes as described above. These dishes were suspended over glass cover slips in closed chambers maintained under the conditions given in Table 2. Twenty-four hours later the slips, mounted in Colley's solution (Colley, 1925), were examined microscopically. The patterns of germination observed are given in Table 2.

Humidities.—The relative humidities within the tightly closed jars were achieved with saturated solutions of the following compounds: Pyrocatechol (94%), KCl (85%), NaCl (75%), $NaNO_2$ (65%), glucose (55%), KNO_2 (48%), NaI (38%), and NaOH (6%). Since a means to measure the actual humidities was not available, the percentages were taken from a table given by Winston and Bates (1960). Distilled H_2O provided a relative humidity of 100%; anhydrous $CaCl_2$ in a dry jar maintained a relative humidity at or near zero percent.

Table 1. Infectivity of *Entomophthora muscae* conidia falling upon healthy *Musca domestica* held at various relative humidities at 21°C.

Relative humidity	No. flies at risk	Post-exposure days with percentage dying of infection					Total percentage fatally infected
		1–4	5	6	7	8	
100	20	0	5	50	45	0	100
94	20	0	5	70	25	0	100
85	21	0	0	86	14	0	100
75	23	0	0	61	30	9	100
65	24	0	0	38	29	12	79
55	22	0	10	36	36	0	82
48	20	0	0	60	0	0	60
38	20	0	10	50	20	0	80
6	21	0	14	33	10	14	71
0	15	0	0	7	13	0	20

Results and Discussion

Flies exposed to conidial showers falling through atmospheres with relative humidities ranging from zero to 100% acquired fatal *E. muscae* infections (Table 1). At relative humidities of 75% and above all flies at risk became infected, while at 65% R.H. and below some flies apparently escaped infection. Why some flies did not acquire the infection is unclear. Inherent resistance is probably not the explanation, since uninfected survivors from these tests later proved to be quite susceptible when exposed to conidial showers at 75% R.H.

Primary conidia were produced and discharged from cadavers of flies held at relative humidities ranging from zero to 100%. Conidial showers from cadavers in atmospheres with low relative humidities (6 and 38%) were markedly scanty compared to those from cadavers at 55% R.H. or higher. That some conidia are produced at 0% R.H. suggests that the cadaver of a well-nourished fly supplies moisture adequate for the development of a few of these asexual spores. The data in Table 2 show that some primary conidia produced secondary conidia at all humidities considered. Often these secondary conidia developed small lateral bulges. Other secondary conidia produced germ tubes about as long as a secondary conidium at humidities of 48% and above. Only at 100% R.H. were germ tubes more than twice as long as a conidium formed from secondary conidia. These long tubes were often branched and sometimes two or more tubes arose from one conidium. The significance of these variations is unknown. Long tubes completely separated from the secondary conidium of origin were observed only at 100% R.H. In no instances were forms clearly identifiable as tertiary conidia observed in any of the slide preparations.

Table 2. Germination patterns among conidia of *Entomophthora muscae* discharged onto glass slips from house-fly cadavers during a 24-hour period at various relative humidities at 21°C.

	Percentages of forms present*						
					Secondaries germinating		
Relative humidity	Primary alone	Primary with secondary	Secondary alone	Secondary with bulge	Germ tube short**	Germ tube long***	Long germ tubes alone
100	27	29	24	0	7	16	7
94	22	21	13	7	37	0	0
85	17	14	29	27	13	0	0
75	16	3	47	23	11	0	0
65	29	8	51	5	7	0	0
55	33	5	43	8	11	0	0
48	41	4	52	1	2	0	0
38	24	11	61	4	0	0	0
6	48	18	33	1	0	0	0
0	73	17	9	1	0	0	0

* Average of four replicates, 100 forms per replication.
** Germ Tube Short = Length no greater than length of conidium.
*** Germ Tube Long = Length greater than length of conidium.

The foregoing results indicate that: 1, conidia falling through atmospheres with relative humidities ranging from zero to 100% do give rise to infections in flies (Table 1); and 2, long germ tubes, the invasive form of the fungus, are produced only at 100% R.H. (Table 2). When viewed together these findings suggest that the relative humidity within the boundary layer surrounding the fly's body is at or very near the saturation point. From an epizootiological viewpoint these findings also suggest that *E. muscae* can be transmitted within house-fly populations during periods of comparatively dry and cool weather. Low humidities in themselves do not prevent the spread of *E. muscae* infections in fly populations.

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